AMENDMENTS TO THE SPECIFICATION

Please delete the abstract on page 36 and replace it with the following rewritten abstract:

The present invention is directed to methods and compositions useful as biosensors that specifically interact with various pathogens and other target analytes. The biosensor itself, comprises functionalized dendritic tethers derivatized for attachment to a variety of surfaces as self-assembled monolayers (SAMs) as well as attached binding moieties (sometimes referred to as capture binding ligands). Accordingly, the present invention provides compositions comprising supports comprising surfaces to which the binding moieties (e.g. antibodies) are attached for the detection of target analytes (e.g. pathogens) as well as methods and compositions relating to the attachment of such binding moieties.

Please delete the paragraph beginning on page 1, line 6 and replace it with the following rewritten paragraph:

In the wake of the September 11, 2001 terrorist attack on the World Trade Center and the Pentagon, and the subsequent contamination of postal centers and other public buildings by anthrax spores and weapons grade anthrax aerosols, with associated deaths of postal workers and a recipient of a cross-contaminated letter, [[~~has~~]] these tragic events have underlined our relative lack of rapid and effective detection protocols for both military and civilian populations. Furthermore, while it is possible to respond to a terrorist attack involving one known pathogen, such as anthrax, it is chilling to envision the potential chaos that might result from the simultaneous exposure of large segments of the general population to a multiplicity of pathogens, whether air-borne, water-borne or food-borne.

Please delete the paragraph beginning on page 1, line 15 and replace it with the following rewritten paragraph:

At the current time, there are no simple recognition systems that are particularly well suited to the simultaneous detection of multiple pathogenic agents. Nor are there rapid, reliable methods to identify the presence of these agents in the field, particularly for use by first responders (police, fire-fighters, paramedics, etc.). The current four-tier laboratory response network,

designed to react to bioterrorism threats, proved woefully slow and cumbersome during the recent anthrax dispersion and hoax testing. For example, the first two tiers alone require at least 48 hours for identification of suspect pathogens. In addition, tiers three and four require even more sophisticated testing than tiers one and two, testing that must occur at more advanced centers, such as the Center for Disease Control and Prevention (CDC) and the US Army Medical Research Institute for Infectious Diseases (USAMRID). What is needed is a system that can be employed at the point of attack, operated by relatively untrained personnel (nonscientists), and that rapidly identifies a variety of bioterrorism agents. A recent report [[~~of~~]] from NIH-NIAID (*NIAID Biodefense Research Agenda for CDC Category A Agents*, Feb. **2002**, National Institutes of Health) has identified a number of pathogens that are ideal bioterrorism agents, for example, tularemia, botulinum toxin, Yersinia pestus (plague), and smallpox. Notably, none of these agents are specifically detectable with currently existing detection systems.

Please delete the paragraph beginning on page 5, line 13 and replace it with the following rewritten paragraph:

In an additional aspect the bacteria is selected from the group consisting of: Bacillus, Vibrio, e.g. V. cholerae; Escherichia, e.g. Enterotoxigenic E. coli, Shigella, e.g. S. dysenteriae; Salmonella, e.g. S. typhi; Mycobacterium e.g. M. tuberculosis, M. leprae; Clostridium, e.g. C. botulinum, C. tetani, C. difficile, C.perfringens; Cornyebacterium, e.g. C. diphtheriae; Streptococcus, S. pyogenes, S. pneumoniae; Staphylococcus, e.g. S. aureus; Haemophilus, e.g. H. influenzae; Neisseria, e.g. N. meningitidis, N. gonorrhoeae; Yersinia, e.g. G. lamblia, Y. pestis, Pseudomonas, e.g. P. aeruginosa, P. putida; Chlamydia, e.g. C. trachomatis; Bordetella, e.g. B. pertussis; and Treponema, e.g. T. palladium.

Please delete the paragraph beginning on page 6, line 9 and replace it with the following rewritten paragraph:

In an additional aspect, the instant invention provides a method of attaching a first compound to a second compound by glycosylation of the first compound with a promiscuous O-linked-glycosyltransferase, followed by oxidation of the glycosylation to produce [[a]] <u>an</u> aldehyde-

derivitized first compound and reacting the aldehyde-derivitized first compound with a hydrazide-derivitized second compound to attach the first compound to the second compound.

Please delete the paragraph beginning on page 7, line 3 and replace it with the following rewritten paragraph:

In an [[~~addition~~]] additional aspect, the instant invention provides a method of atomic force microscopy employing a composition comprising a terminal dendrimer comprising at least two attachment moieties as part of a linker comprising at least one hydrophilic polymer and at least one rigidity component, as well as a functional moiety.

Please delete the paragraph beginning on page 7, line 7 and replace it with the following rewritten paragraph:

In an [[~~addition~~]] additional aspect, the instant invention provides a method of surface plasmon resonance employing a composition comprising a terminal dendrimer comprising at least two attachment moieties as part of a linker comprising at least one hydrophilic polymer and at least one rigidity component, as well as a functional moiety.

Please delete the paragraph beginning on page 7, line 11 and replace it with the following rewritten paragraph:

In an [[~~addition~~]] additional aspect, the instant invention provides a method of quartz crystal microbalance detection employing a composition comprising a terminal dendrimer comprising at least two attachment moieties as part of a linker comprising at least one hydrophilic polymer and at least one rigidity component, as well as a functional moiety.

Please delete the paragraph beginning on page 9, line 18 and replace it with the following rewritten paragraph:

Accordingly, the present invention provides compositions and methods for detecting the presence or absence of target analytes, such as the pathogens described above, in a sample. As will be appreciated by those in the art, the sample solution may comprise any number of things,

including, but not limited to, bodily fluids (including, but not limited to, blood, urine, serum, lymph, saliva, anal and vaginal secretions, perspiration and semen, of virtually any organism, with mammalian samples being preferred and human samples being particularly preferred); environmental samples (including, but not limited to, air, agricultural, water and soil samples); biological warfare agent samples; research samples; and raw samples (bacteria, virus, genomic DNA, etc.); As will be appreciated by those in the art, virtually any experimental manipulation may have been done on the sample.

Please delete the paragraph beginning on page 11, line 4 and replace it with the following rewritten paragraph:

Of particular relevance to the biosensor compositions and methods of the instant invention are a variety of pathogens outlined in a the recent report by NIH-NIAID cited above. Each the cited pathogens could be employed as bioterror weapons against military and civilian targets. These potential bioterror agents include: Bacillus anthracis (anthrax) toxin, Yersinia [[~~pesius~~]] pestus (plague), botulinum toxin, tularemia (Francisella tularensis) and smallpox virus.

Please delete the paragraph beginning on page 11, line 21 and replace it with the following rewritten paragraph:

Furthermore, the technology of the instant invention is capable of detecting bioengineered pathogens. Such detection only requires that a specific binding moiety for the bioengineered pathogen be isolated and immobilized on a dendritic [[~~tethers~~]] tether as a SAM for use in AFM or in an SPR or QCM detector. For example, an antibody specific to a particular bioengineered pathogen could be generated, using methods well known in the art, and attached to the derivitized dendritic tethers of the instant invention and used for the detection of that pathogen.

Please delete the paragraph beginning on page 12, line 6 and replace it with the following rewritten paragraph:

In addition to the pathogens described above, many other viruses [[~~that~~]] may be detected using the biosensors of the instant invention. Such viruses include, but are not limited to,

orthomyxoviruses, (e.g. influenza virus), paramyxoviruses (e.g respiratory syncytial virus, mumps virus, measles virus), adenoviruses, rhinoviruses, coronaviruses, reoviruses, togaviruses (e.g. rubella virus), parvoviruses, poxviruses (e.g. variola virus, vaccinia virus), enteroviruses (e.g. poliovirus, coxsackievirus), hepatitis viruses (including A, B and C), herpesviruses (e.g. Herpes simplex virus, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus), rotaviruses, Norwalk viruses, hantavirus, arenavirus, rhabdovirus (e.g. rabies virus), retroviruses (including HIV, HTLV-I and -II), papovaviruses (e.g. papillomavirus), polyomaviruses, and picornaviruses, and the like. Examples of the wide variety of pathogenic and non-pathogenic prokaryotes amenable to detection by the instant invention are, Bacillus, Vibrio, e.g. V. cholerae; Escherichia, e.g. Enterotoxigenic E. coli, Shigella, e.g. S. dysenteriae; Salmonella, e.g. S. typhi; Mycobacterium e.g. M. tuberculosis, M. leprae; Clostridium, e.g. C. botulinum, C. tetani, C. difficile, C.perfringens; Cornyebacterium, e.g. C. diphtheriae; Streptococcus, S. pyogenes, S. pneumoniae; Staphylococcus, e.g. S. aureus; Haemophilus, e.g. H. influenzae; Neisseria, e.g. N. meningitidis, N. gonorrhoeae; Yersinia, e.g. G. lamblia, Y. pestis, Pseudomonas, e.g. P. aeruginosa, P. putida; Chlamydia, e.g. C. trachomatis; Bordetella, e.g. B. pertussis; Treponema, e.g. T. palladium; and the like.

Please delete the paragraph beginning on page 13, line 12 and replace it with the following rewritten paragraph:

The dendrimeric compositions of the present invention [[~~general~~]] generally comprise three components: a terminal dendrimer, used ultimately to attach to a surface using at least two attachment moieties; a linker, optionally comprising at least one hydrophilic polymeric section and a rigidity component; and a functional moiety used for attachment of a binding moiety (sometimes referred to herein as a "binding moiety" or a "capture moiety").

Please delete the paragraph beginning on page 17, line 8 and replace it with the following rewritten paragraph:

For a typical biosensor construct, the dendritic tether illustrated in Figure 2 is attached to a surface as a self-assembled monolayer (SAM). By "monolayer" or "self-assembled monolayer" or "SAM" herein is meant a relatively ordered assembly of molecules spontaneously

chemisorbed on a surface, in which the molecules are oriented approximately parallel to each other and roughly perpendicular to the surface. A majority of the molecules includes a functional group that adheres to the surface, and a portion that interacts with neighboring molecules in the monolayer to form the relatively ordered array. A "mixed" monolayer comprises a heterogeneous monolayer, that is, where at least two different molecules make up the monolayer. For example, in the present invention, one SAM species can comprise the dendrimeric composition, and a second species can comprise an alkyl chain, or a hydrophilic polymer species similar to that contained in the dendrimeric composition.. Previous self-assembled monolayer constructs have been used in a variety of formats, primarily with single-stranded long-chain alkylthiols having a single attachment functionality for surface plasmon resonance measurements and some designed for molecular electronics applications (V. Chechik, R. M. Crooks and C. J. M. Stirling, "Reactions and Reactivity in Self-Assembled Monolayers", Adv. Mater. **2000**, *12*, 1161; S. Flink, F. C. J. M. van Veggel and D. Reinhoudt, "Sensor Functionalities in Self-Assembled Monolayers", Adv. Mater. **2000**, *12*, 1315; T. Neumann, M.-L. Johansson, D. Kambhampati and W. Knoll, "Surface-Plasmon Fluorescence Spectroscopy", Adv. Funct. Mater. **2002**, *12*, 575. However, monodisperse multi-arm dendrimers with multiple attachment functionalities can provide greatly enhanced structural stability in SAMs. Previous work by other groups have described SAMs on gold consisting of pre-formed [[~~fouth~~]] fourth generation poly(amido-amine) (PAMAM) dendrimers having terminal groups functionalized with thiol groups by addition of mercaptodecanoic acid. Such SAMs have distinct disadvantages because they consist of commercial dendrimers that are not [[~~mondisperse~~]] monodisperse (they are mixtures). Consequently these dendrimers contain a varying number of terminal functionalities leading to the formation of irregular surfaces. In addition to being imperfect, and not readily tailorable to specific bioactive molecules, they must be tethered to a long alkyl chain associated with the gold surface. The necessary use of this long tether dictates that the active site is actually quite far from the gold sensory surface, leading to a viscoelastic effect on a quartz crystal microbalance (See Ref. 3, Section D. "The Behavior of Dendrimers on Surfaces and in Amphiphilic Materials", and references therein.) and to nonlinear readings on a surface plasmon resonance sensor. (C. Nylander, B. Liedberg and T. Lind, "Gas Detection by Means of Surface Plasmon Resonance", Sensors and Actuators, **1982/3**, *3*, 79; C. Jeppesen, J. Y. Wong, T. L. Kuhl, J. N. Israelachvili, N. Mulach, S. Zaplipsky and C. N. Marques, "Impact of Polymer

Tether Length on Multiple L:igand-Receptor Bond Formation", Science, **2001**, *293*, 265.) Jeppesen et al. have most recently described the impact and significance of the length and configuration of the tether groups on ligand-bond formation. Their detailed analysis of the implications of using flexible long single-chain tethers, by Monte Carlo simulations, diffusion reaction theory, and surface force measurements, lead to the startling conclusion that the tether groups do not usually exist in the highly extended configurations necessary for efficient binding between, for example, biotin attached to the tether, and [[~~strepavidin~~]] streptavidin (a common construct used in biosensors). While this dictates that the tether can stretch to attach to a target, it also points to the fact that the more rigid dendrimer scaffolds of the instant invention provide a faster and more effective binding scenario by eliminating the conformational re-ordering that is a necessary initial step with more flexible tethers. This analysis holds out that increased speed and sensitivity in target binding is possible by designing order and "rigidity" into the detecting surface as is described above with regard to the dendritic tethers of the instant invention.

Please delete the paragraph beginning on page 26, line 3 and replace it with the following rewritten paragraph:

Another analytical [[~~toe~~]] tool useful in investigating binding interactions on surfaces is surface plasmon resonance. (See Lahiri et al., Analytical Chemistry, 71(4) **1999** 777-790.) SPR is particularly useful in that it allows detection of interactions in real-time. In general, SPR is carried out by measuring changes in the refractive index of a medium in close proximity to a thin film deposited on a substrate. Specifically, the resonance angle (1_m), which corresponds to the angle of minimum intensity of reflected light, can be altered by changes in the refractive index of the medium. (See, Raether, H., Physics of Thin Films, Hass et al., Eds. **1977** 145-261; Stenberg et al., J. Colloid Interface Sci., 143 **1991** 513-526.) These changes in refractive index are initiated by alterations to the local environment, e.g. by anti-ligands binding to ligands attached to the surface of the film. Films useful in SPR can be comprised of binding moieties coupled to SAM forming organic molecules. In addition, organic compounds containing thiol groups are particularly preferred in order to simplify attachement of the film to a substrate.

Please delete the paragraph beginning on page 27, line 12 and replace it with the following rewritten paragraph:

The following is an example of the technology as applied for the detection of *Bacillus anthracis*. As described above, the approach to pathogen detection of the instant application could indeed be regarded as universal by coupling of appropriate binding moieties [[~~could be immobilized~~]] to dendrimer SAMs.

Please delete the paragraph beginning on page 27, line 16 and replace it with the following rewritten paragraph:

In this example, the binding moiety to be coupled to the dendritic tethers of the instant invention is an antibody against a recombinant antibody against anthrax protective antigen (PA), a necessary component of anthrax toxin, based on a monoclonal antibody originally developed by Dr. S. Leppla (NIAID, NIH). A DNA plasmid containing the gene for this binding moiety was obtained from Prof. Georgiou (Univ. Texas – Austin). The recombinant antibody consists of a pair of variable regions that specifically recognize PA with high affinity, tied together by a repeating peptide $(GlyGlyGlyGlySer)_3$. Standard methods of gene expression allow the production <u>of</u> large quantities of recombinant antibody protein.

Please delete the paragraph beginning on page 30, line 1 and replace it with the following rewritten paragraph:

In order to remove the high substrate specificity associated with OGT, the [[~~nuceloties~~]] <u>nucleotides</u> encoding the specificity domain were excised from the gene encoding the enzyme. The modified protein was then sequenced to verify the modification, expressed, isolated and purified. Excision of a portion of the native binding domain renders the enzyme sufficiently promiscuous to glycosylate hydroxyl group residues in a wider variety of amino acid sequences. Glycosylation of the anti-PA recombinant antibody protein is demonstrated by the colorimetric assay and by MALDI. To prevent glycosylation of the antibody's active site, it is reacted with PA immobilized on an affinity resin. Once glycosylation has been accomplished, the glycosylated recombinant antibody is separated from immobilized antigen in a high-salt buffer

and the antibody separated from immobilized antigen and enzyme by filtration. Subsequent oxidation of the sugar(s) is followed by attachment to a hydrazide-derivatized dendritic tether. Attachment is accomplished by allowing the aldehyde-derivatized antibody to react with the hydrazide-derivatized tether for six hours at room temperature in solution or, more conveniently, by recycling the prepared antibody *in situ* over a previously prepared dendritic SAM.